SUPPLEMENTAL MATERIAL

Expanded Methods

Flow Cytometry: For circulating T cell analysis, total leukocytes were isolated from heparinized blood and red cells were lysed osmotically. The remaining leukocytes were centrifuged (800 x g), washed twice with PBS and containing 0.5 % BSA (staining buffer). The cells were then counted and one million cells re-suspended in 1 % BSA PBS and stored on ice less than 30 minutes. The cells were then stained for 15 minutes at 4°C with fluorescently-labeled antibodies described below, washed twice and re-suspended in staining buffer and analyzed immediately.

To analyze leukocytes in aorta, spleen and lymph nodes, these respective tissues were digested using collagenase type IX (125u/ml); collagenase type IS (450U/ml) and hyaluronidase IS (60U/ml) dissolved in 20 mM HEPES-PBS buffer containing calcium and magnesium for 30 minutes at 37^{o} C, while constantly agitated. The dissolved tissue was then passed through a 70 μm sterile filter (Falcon, BD), yielding a single cell suspension. Cells were washed twice with staining buffer and additionally incubated for 30 minutes in 37^{o} C with RPMI supplemented with 10% FCS, then washed again, counted, stained and analyzed using multi color flow cytometry as described above.

Flow cytometry was performed using a LSR-II flow cytometer with DIVA software (Becton Dickinson) on both circulating and tissue-derived cells using similar techniques except that T cells in blood were analyzed within the entire PBMC gate. For aortic single cell suspensions, an initial gate was applied to exclude cell debris from further analysis, and CD45 positive cells were identified as leukocytes within the aortic cell suspension. T cells were identified with anti-CD3 antibodies. B cells were identified by positive CD19 staining. Cells co-staining for I-A^{b} and CD11c were identified as dendritic cells and CD11b and I-A^{b} positive cells were considered macrophages. NK 1.1 positive staining identified natural killer cells. Gating was applied using fluorescence minus one (FMO) controls constructed with staining

panels where one of the fluorescent markers of interest was replaced with an isotype control. Data were analyzed with Flowjo software (Treestar).

Materials: CTLA4-Ig (Abatacept, Orencia) was obtained commercially and prepared freshly each week according to manufacturer's instructions. Control IgG was obtained from eBioscience. Osmotic minipumps (Models 2002 and 2004) were from Alzet Corporation. Angiotensin II and chemicals for buffers were obtained from Sigma. Cell culture media was from Cell-Gro. Antibodies for staining were from BD Pharmingen and were used in different multi-color combinations: FITC anti-CD45 (30-F11); PerCP anti CD45 (30-F11); APC anti CD19 (1D3); PE anti CD4 (GK1.5); APC ANTI CD4 (GK1.5); FITC anti CD4 (gk 1.5); PerCP anti CD8 (53-6.7); APC anti CD3 (145-2C11); PE anti I-A^b (AF6-120.1); FITC anti I-A^b (AF6-120.1); APC anti CD11c (HL3); APC CD11b (M1/70); PE 62L (MEL-14); APC CD4 (RM4-5); PerCP CD4 (RM4-5); PE CCR5; FITC g/d (GL3); FITC Vbeta7; FITC CD44 (IM7); FITC CD69 (H1.2F3); PE CD19 (1D3); PE NK1.1 (PK136); PE CD86 (GL1); FITC CD80 (16-10A1), FITC MHC II (2G9).

Supplementary Table 1. Tail cuff systolic blood pressures measured before and after treatment.

	Systolic Blood Pressure Mean (mmHg)				
	n	**Pre-treatment (Day 0)**	**Post-treatment (Day 14 or 21)**	**Mean difference**	**95% CI of Difference**
Ang II-induced hypertension					
Sham + Vehicle	5	117	118	0.2	-10 - 10
Sham + CTLA4-Ig	5	116	115	-0.7	-10 - 9
Ang II + Vehicle	5	116	153 *†	36	24 - 47
Ang II + CTLA4-Ig	5	117	135 *†‡	20	10 - 31
WT - Sham	7	121	117	3	-14 - 7
$B7^{-/-}$ - Sham	11	116	111	5	-14 - 3
WT + Ang II	6	117	154 *†	38	26 - 49
$B7^{-/-}$ + Ang II	12	112	124 *†‡	12	4 - 20
Irradiated $B7^{-/-}$ mice					
Sham + BMT	4	110	100	10	-24 - 4
Ang II + BMT	6	109	144 *§	36	24 - 47
DOCA-salt induced hypertension					
Sham + Vehicle	10	116	121	4	-4 - 13
Sham + CTLA4-Ig	10	119	125	6	-3 – 14
DOCA + Vehicle	12	116	153 *†	37	29 – 45
DOCA + CTLA4-Ig	13	117	135 *†‡	18	10 - 26

* P<0.001 Vs Pre-treatment; † P<0.001 Vs Corresponding Sham treatment, ‡ P<0.001 Vs Ang II/DOCA + Vehicle or WT + Ang II; § P<0.01 Vs $B7^{-/-}$ + Ang II

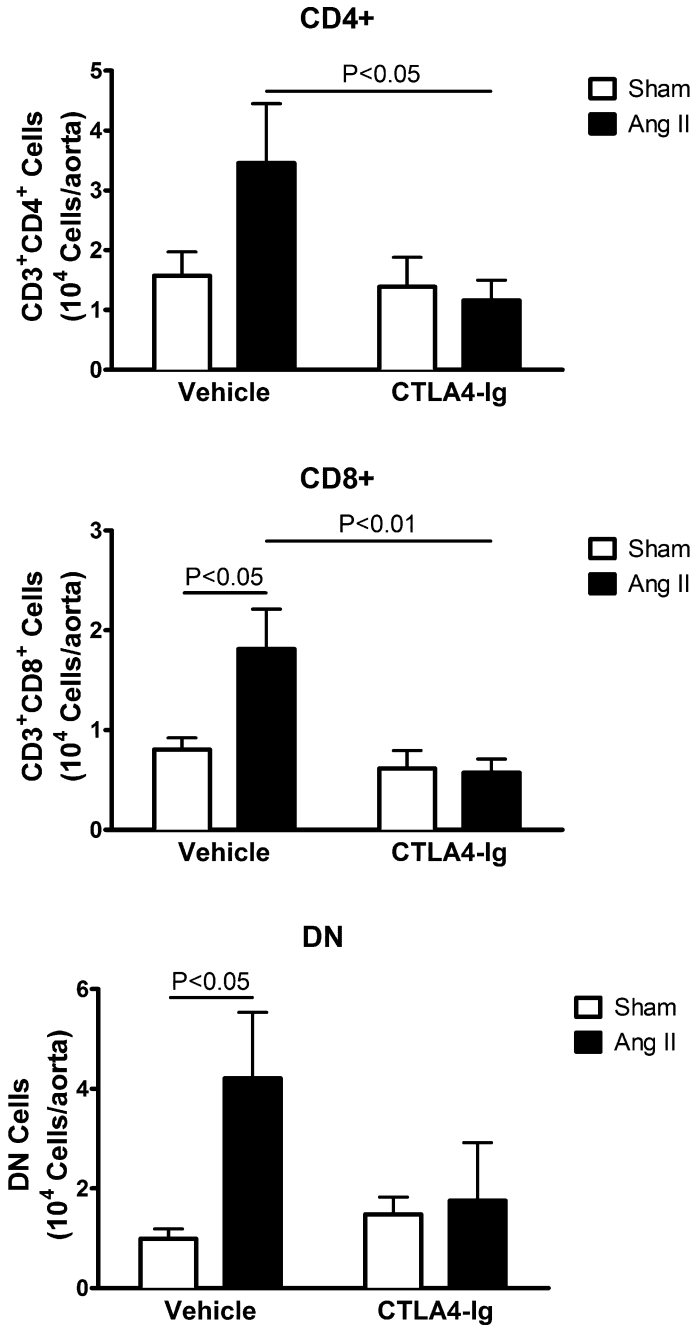

Figure 1: CTLA4-Ig inhibits angiotensin II-induced infiltration of T cell subsets. Absolute numbers of aortic (A) $CD4^+$, (B) $CD8^+$ and (C) double negative (DN) T cells from all treatment groups. (n=6-8). Comparisons were made using two-way ANOVA and statistical values reflect Bonferonni correction.

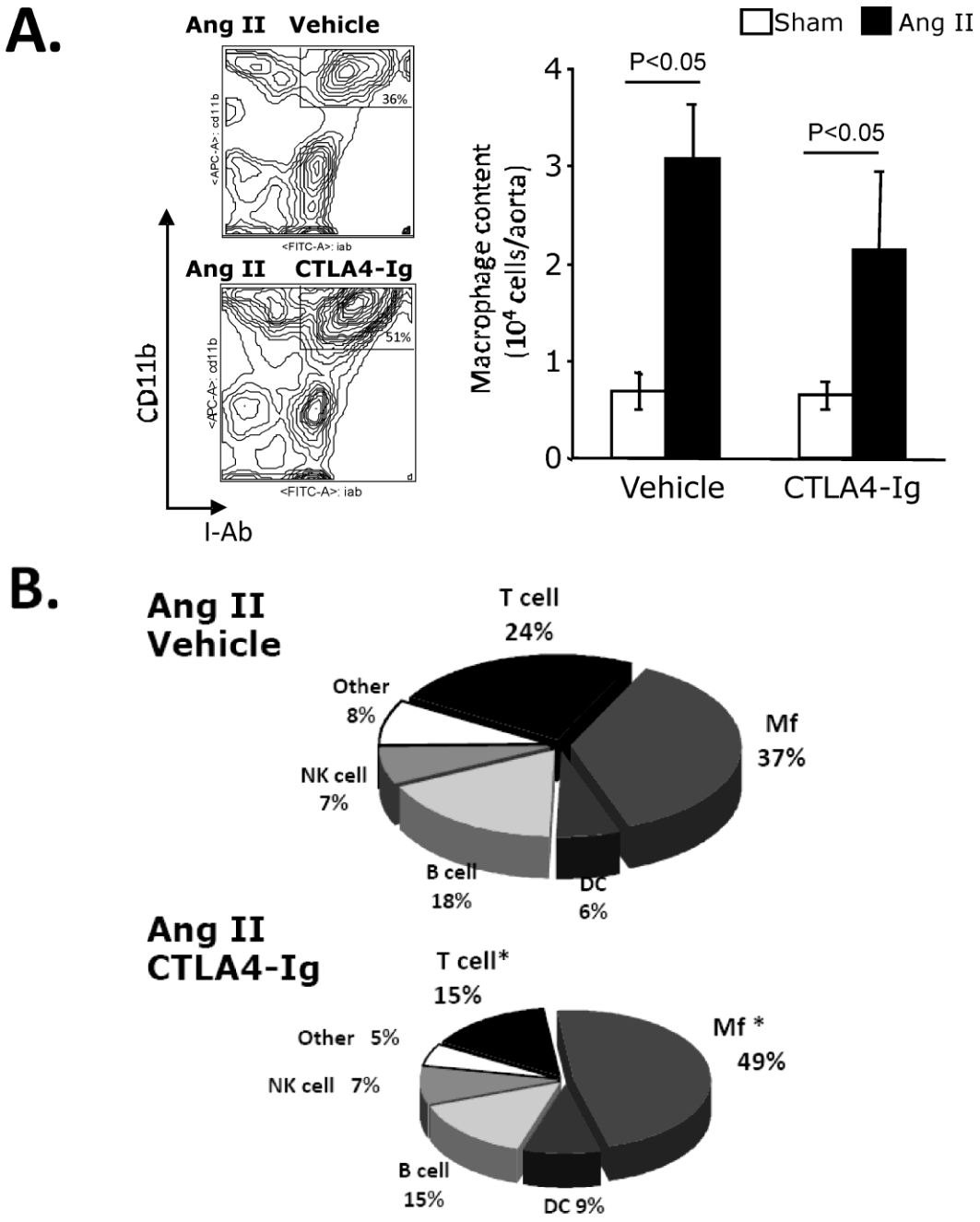

Figure 2. CTLA4-Ig does not affect aortic leukocyte populations. (A) Representative plots and absolute numbers of macrophages ($CD45^+CD11b^+I\text{-}Ab^+$ cells) in the aortas of either saline or CTLA-4Ig treated mice with angiotensin II-dependent hypertension. (n=6-8). Comparisons were made using two-way ANOVA and statistical values reflect Bonferonni correction. (B) Pie charts showing immune cell composition in aortas of mice treated with angiotensin II and either vehicle or CTLA4-Ig (n=7). * $P < 0.01$ vs. Vehicle.

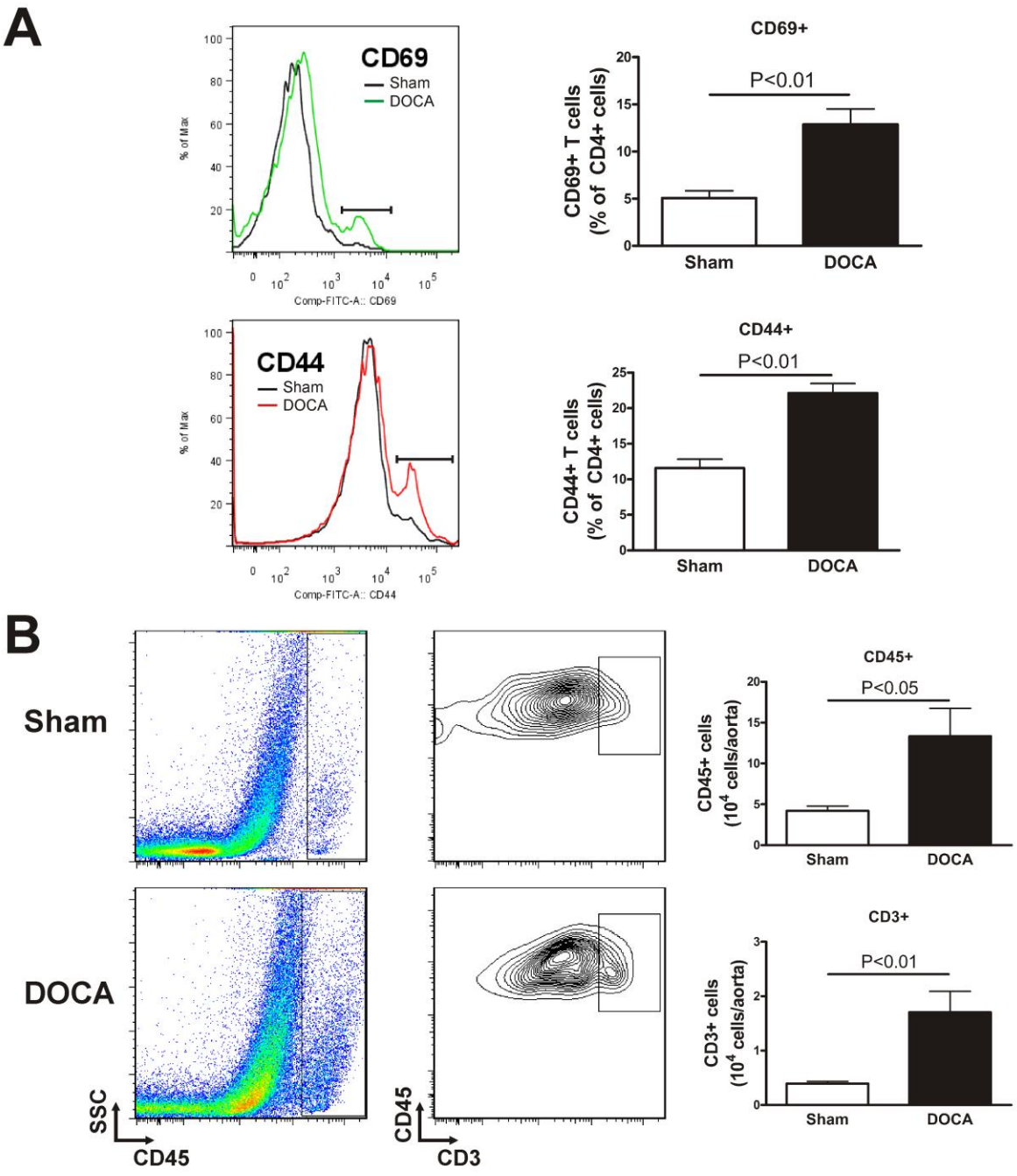

Figure 3. Two week DOCA-salt treatment induces T cells activation and vascular infiltration. (A) Representative histograms and mean data comparing the effect 2 week DOCA salt treatment on $CD4^+$ T cell surface expression of the early activation antigen CD69 and CD44. (B) Representative plots and mean data of aortic infiltration of total leukocytes ($CD45^+$) and T cells ($CD3^+$) after 2 weeks of DOCA-salt treatment. (n=5 for all groups). Comparisons were made using two-tailed t-tests.